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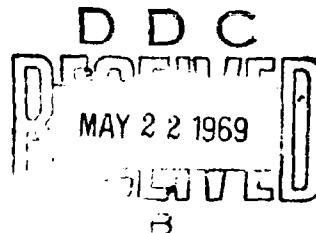
EATR 4276

THE EFFECTS OF ATROPINE-OXIME THERAPY ON
CHOLINESTERASE ACTIVITY AND THE SURVIVAL
OF ANIMALS POISONED WITH DIAZINON

by

Larrel W. Harris
Joseph H. Fleisher
Terry A. Innerebner
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Van M. Sim

April 1969

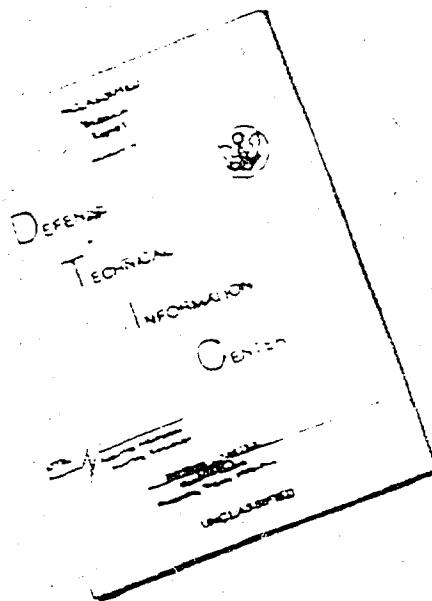


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Task 1B662706A09709

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FOREWORD

The work described in this report was authorized under Task 1B662706A09709, Medical Defense Aspects of Chemical Agents, Lethal Agents (U). The work was started in March 1967 and was completed March 1968. The experimental data are recorded in notebook MN 1808.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animals Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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DIGEST

This work was undertaken to determine the effectiveness of atropine +2-PAMCl treatment of animals poisoned with Diazinon, an organophosphate requiring metabolic activation prior to becoming an inhibitor of Cholinesterase (ChE).

Inhibited diaphragm ChE in rats poisoned with this organophosphate and treated 24 hours later with 2-PAMCl showed significant reactivation regardless of whether the oxime was administered by the oral or intravenous route. However, 2-PAMCl given with intramuscular atropine to rats intoxicated with Diazinon was significantly more effective by the oral route than when injected intravenously.

Our findings suggest that effective treatment of Diazinon intoxication requires the maintenance of a level of oxime high enough to reactivate inhibited ChE during the time in which active inhibitor is formed. This can be more readily achieved when 2-PAMCl is given orally following an initial intravenous dose in conjunction with atropine given intramuscularly.

CONTENTS

| | Page |
|---|------|
| I. INTRODUCTION | 7 |
| II. EXPERIMENTATION..... | 7 |
| A. Materials | 7 |
| B. Animals | 8 |
| C. Methods | 8 |
| III. RESULTS | 11 |
| A. Changes in Brain and Diaphragm ChE in Rats After Poisoning With Diazinon | 11 |
| B. Aging of Rabbit Brain AChE | 11 |
| C. Antagonism of Diazinon Poisoning | 12 |
| D. Reactivation of ChE by 2-PAMCl <i>In Vivo</i> | 13 |
| E. Blood ChE Activity and Survival in Rabbits Treated With Atropine and 2-PAMCl | 13 |
| IV. DISCUSSION | 13 |
| V. CONCLUSIONS | 15 |
| LITERATURE CITED | 17 |
| DISTRIBUTION LIST | 19 |

LIST OF FIGURES

| Figure | | Page |
|--------|--|------|
| 1. | Aging of Brain Acetylcholinesterase (AChE) in Rats Poisoned With Diazinon | 9 |
| 2. | Changes in Whole Blood ChE Activity in Rabbits Poisoned With Diazinon and Treated With PAM | 10 |

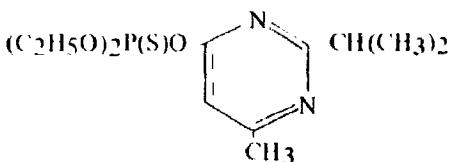
LIST OF TABLES

| Table | | Page |
|-------|---|------|
| I. | Cholinesterase Activity of Rat Brain and Diaphragm Homogenates at Various Times After Poisoning With Diazinon | 11 |
| II. | The Effects of Various Treatments on the LD ₅₀ of Diazinon in Rats | 12 |
| III. | The Effect of 2-PAMCl on Diaphragm ChE Activity in Rats Poisoned With 0.8 LD ₅₀ of Diazinon | 13 |

THE EFFECTS OF ATROPINE OXIME THERAPY
ON CHOLINESTERASE ACTIVITY AND THE SURVIVAL
OF ANIMALS POISONED WITH DIAZINON

I. INTRODUCTION.

This study was undertaken to provide information on the antidotal effectiveness of pyridine-2-aldoxime methochloride (2-PAMCl) against Diazinon (**I**) poisoning in animals in view of conflicting reports concerning its usefulness against this organophosphate.^{1,2}



O,O-Diethyl O-(2-isopropyl-6-methyl-4-pyrimidyl) phosphorothionate

One possible mechanism limiting the effectiveness of 2-PAMCl would be the gradual conversion of Diazinon-inhibited cholinesterase (ChE) to a state resistant to reactivation by nucleophilic compounds like 2-PAMCl, a phenomenon called "aging."³

Metabolic activation of Diazinon to a more potent anticholinesterase *in vitro* has been reported.^{4,5} The fact that toxic signs in rats given Diazinon are slow to appear² may be a reflection of such metabolic activation of Diazinon. If this occurs *in vivo* in a manner analogous to parathion,⁶ a single intravenous injection of 2-PAMCl given at the first sign of poisoning might be only temporarily effective because the Diazinon would continue to be activated beyond the time that an adequate concentration of oxime would be present. The oral route, yielding more prolonged blood levels of oxime,^{7,8} would be more effective.

The relative ineffectiveness of 2-PAMCl alone in experimental animals given Diazinon suggested that the effect of 2-PAMCl administered adjunctively with atropine be studied. The rate of inhibition and spontaneous recovery of ChE in animals poisoned with Diazinon, the rate of conversion of the phosphorylated ChE to a form resistant to oxime therapy, and the therapeutic activity of atropine sulfate and 2-PAMCl were studied with the above considerations in mind.

II. EXPERIMENTATION.

A. Materials.

2-PAMCl and atropine sulfate were obtained from commercial sources. The oxime was dissolved in 0.9% NaCl as needed. The resulting solution was brought to approximate neutrality with NaOH. Atropine sulfate was prepared in distilled water. Technical Diazinon, Batch No. FL6199 and 91.9% pure, was kindly supplied by Geigy Chemical Corporation, Ardsley, N. Y., and was dissolved in peanut oil just before use.

B. Animals.

Female albino rats (110 to 140 grams) and rabbits (2.2 to 2.6 kg) were used. Diazinon and 2-PAMCl were administered to the rats in volumes of 2.5 ml/kg, and atropine was injected in 1 ml/kg doses. All solutions were given to the rabbits in 1 ml/kg doses.

C. Methods.

1. Enzyme Assays

Brain and diaphragm ChE activity was measured in rats at various intervals after poisoning with 234 mg/kg (0.8 LD₅₀) Diazinon orally. To reduce mortality at this dose, the animals were given atropine intramuscularly (im) 10 minutes after poisoning. After sacrificing, the brains and diaphragms were removed, washed, blotted, weighed, and homogenized in distilled water to yield a 10% (fresh weight) homogenate. Each brain homogenate was further diluted with a solution of 0.3M NaCl and 0.05M phosphate buffer at pH 7.4 to yield a 2% homogenate. The diaphragm homogenates were diluted in the same buffered medium to a final concentration of 5%. The acetylcholinesterase (AChE) activity of brain was measured colorimetrically by incubating 1 ml of the brain suspension for 30 minutes at 25°C with 1 ml of 0.006M acetyl-β-methylcholine (mecholyl) prepared in the same buffer. The ChE activity of the diaphragm was measured by incubating 1 ml of homogenate with 1 ml 0.004M acetylcholine chloride (ACh) for 28 minutes at 37°C. The method for assay of enzyme activity has been described.⁹

2. Aging.

The rate of conversion of the inhibited enzyme to a state resistant to oxime treatment was studied by poisoning rats with 0.8 LD₅₀ of Diazinon orally. After the rats were sacrificed at time intervals shown in figure 1, 20% brain homogenates were prepared in H₂O. An 0.8-ml aliquot of homogenate was incubated with 0.2 ml of 10⁻¹M monoisonitrosoacetone (MINA) in 0.25M phosphate buffer, pH 7.8, for 1 hour at 25°C to determine reactivability of inhibited AChE. A second aliquot of the same homogenate was incubated with buffer alone to obtain, as a control, the level of residual AChE activity. The homogenates were then diluted to a 2% concentration. The AChE activity was then measured by the method described above.

3. Reactivation of Inhibited Diaphragm ChE by 2-PAMCl *in Vivo*.

Rats were poisoned orally with 0.8 LD₅₀ of Diazinon followed by administration of 16 mg/kg atropine (im). After 24 hours these rats were divided into three groups: Group I received 30 mg/kg 2-PAMCl intravenously (iv), Group II received 30 mg/kg 2-PAMCl orally, and group III was untreated and set aside as poisoned controls. One hour after 2-PAMCl treatment the animals were sacrificed and their diaphragms removed. The diaphragm ChE activity of each group was measured as described above.

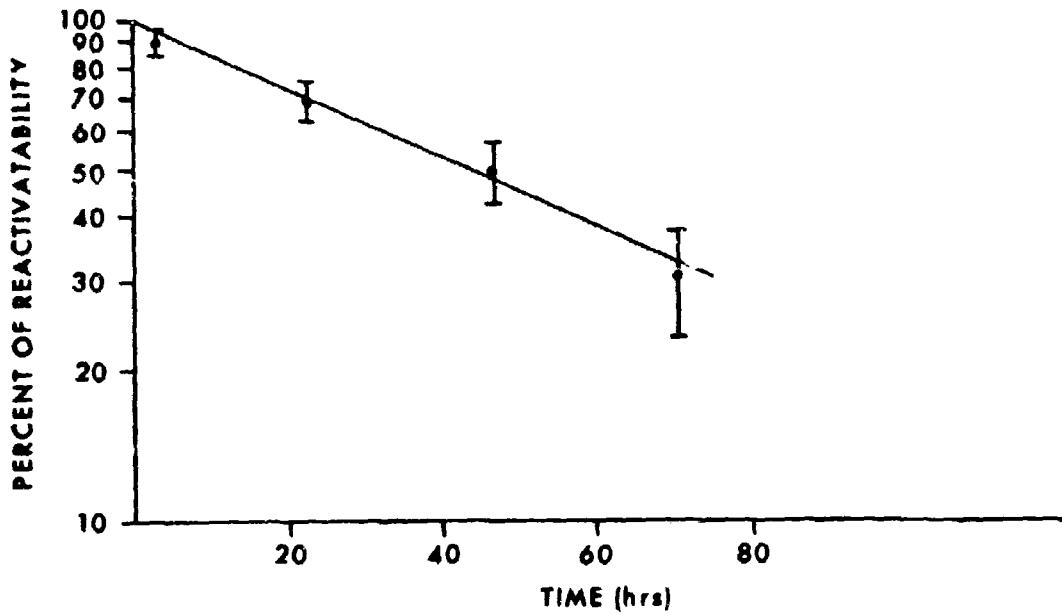


Figure 1. Aging of Brain Acetylcholinesterase (AChE) in Rats Poisoned With Diazinon

4. Toxicity and Therapy.

All estimates of the LD₅₀ of Diazinon in rats and rabbits were based on 24-hour mortalities in groups of six rats and four rabbits given four dose levels having a constant logarithmic increment between successive doses. The LD₅₀ was found by the method of moving averages developed by Thompson (1947)¹⁰ and the mortality tables constructed by Weil (1952).¹¹

5. Changes in Whole Blood ChE Activity and Survival in Poisoned Rabbits Treated With Atropine and 2-PAMCl.

Rabbits (2.0 to 2.5 kg) were used in this study. Blood samples were taken from the vein (or artery) of either ear with a heparinized syringe. After a control blood sample had been removed, each rabbit was given 1600 mg/kg Diazinon* subcutaneously (sc). At the first signs

*The sc LD₅₀ of Diazinon in female rabbits was found to be 670.0 (95% confidence limits: 474.3 to 946.3) mg/kg.

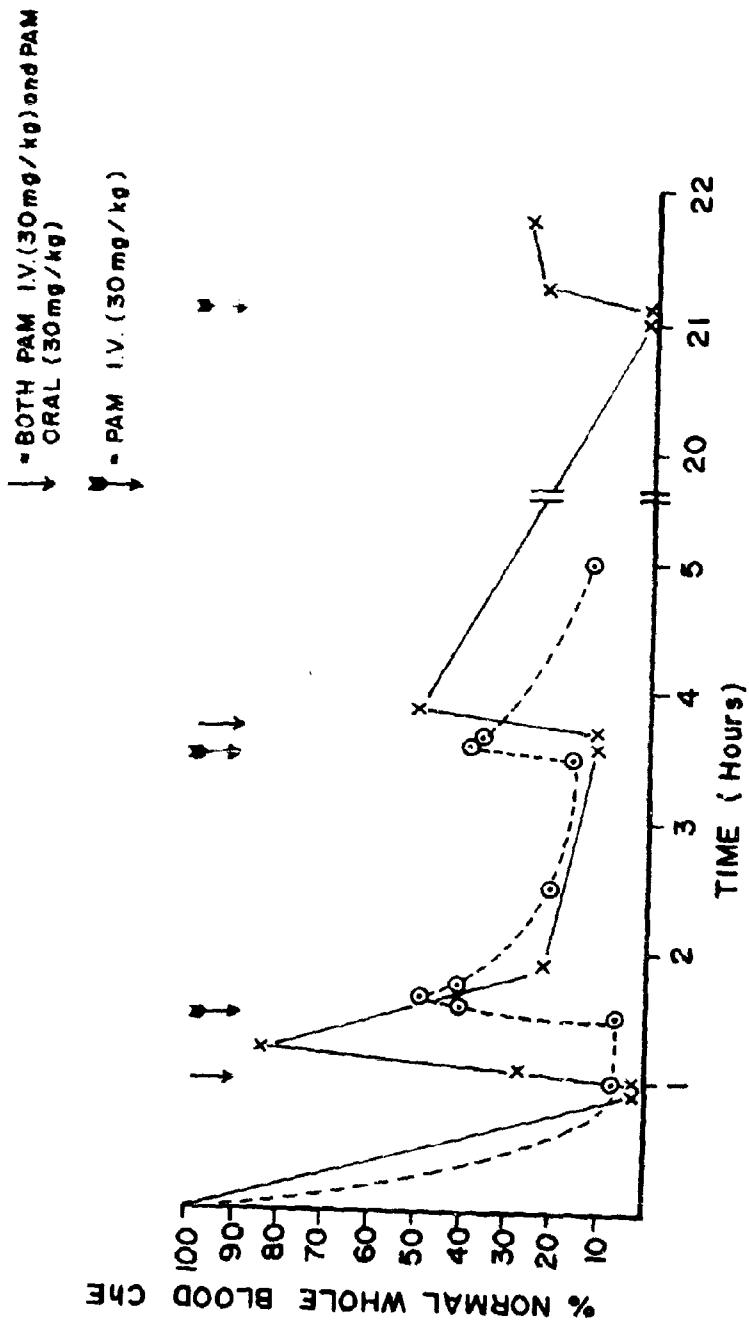


Figure 2. Changes in Whole Blood ChE Activity in Rabbits Poisoned With Diazinon and Treated With PAM
 The solid line represents changes in ChE activity after administration of PAM intravenously and orally;
 the broken line represents changes in ChE activity after intravenous PAM alone.

of poisoning, 16 mg of atropine were injected im. Samples of blood were taken after poisoning and at various time intervals after treatment with oxime, as shown in figure 2. The whole blood ChE activity was assayed in the manner described by Fleisher et al.⁹ After the last blood sample for the day had been taken, animals were given 16 mg atropine im and placed in cages with food and water. The next morning blood samples were taken from surviving animals before and after oxime treatment to ascertain the whole blood ChE activity.

III. RESULTS.

A. Changes in Brain and Diaphragm ChE in Rats After Poisoning With Diazinon.

Changes in enzyme levels of both tissues are summarized in table I. The ChE activity of both tissues gradually recovers, but in the case of diaphragm ChE, the recovery begins only after 24 hours. The enzyme activity of the diaphragm increases from 15.5% of the level in unpoisoned control animals at 24 hours after poisoning to 62.6% 140 hours after poisoning. The AChE activity of the brain also recovers but to a lesser degree during the same time interval.

Table I. Cholinesterase Activity of Rat Brain and Diaphragm Homogenates^a at Various Times After Poisoning With Diazinon

| Time After Poisoning (hours) | Percent of Control ChE Activity (p = 0.05) | |
|------------------------------------|---|------------|
| | Diaphragm | Brain |
| 3 | 22.7 ± 7.8 | 21.6 ± 8.4 |
| 24 | 15.5 ± 6.3 | 22.6 ± 4.3 |
| 48 | 27.2 ± 7.5 | 26.6 ± 4.8 |
| 72 | 40.1 ± 3.0 | 32.3 ± 8.4 |
| 92 | 55.0 ± 7.2 | 45.5 ± 7.4 |
| 116 | 64.0 ± 7.7 | 41.2 ± 4.9 |
| 140 | 62.6 ± 7.5 | 44.8 ± 8.9 |

^aSix animals were used for each time interval in the case of brain ChE and 10 animals in the case of diaphragm ChE.

B. Aging of Rabbit Brain AChE.

The mean values for the percentage of brain AChE reactivatable by MINA were calculated and plotted as a function of time elapsing between the injection of Diazinon and sampling into MINA (figure 1). The decrease in reactivatability is consistent with first-order kinetics over the time intervals shown. The half-time for aging was estimated by interpolation from the curve to be 42.0 (37.0 to 51.0; P = 0.95) hours for brain AChE.

C. Antagonism of Diazinon Poisoning.

The toxicity of Diazinon administered orally to rats with and without various treatments is presented in table II. Rats receiving 1 LD₅₀ (293.8 mg/kg) showed slight to moderate signs 1 to 2 hours after poisoning. At higher dose of Diazinon, the animals showed signs of poisoning within 10 to 15 minutes. Consequently, 10 minutes was chosen for the first administration of therapeutic compounds. Administration of atropine im or 2-PAMCl iv alone 10 minutes after poisoning provided little or no protection (table II). A single dose of 2-PAMCl alone administered orally resulted in more protection, as measured by elevation of the LD₅₀, but not significantly more than that of atropine alone. When a single dose of oxime was given iv adjunctively with atropine im or again a second time iv 4 hours after poisoning, either alone or with atropine, protection was not significantly greater than with a single dose of atropine and 2-PAMCl. When a single dose of the oxime was given orally in conjunction with atropine im, or followed by a subsequent dose of 2-PAMCl administered either orally or iv, with or without atropine, the LD₅₀ was increased to between 2.7 and 3.7 times the LD₅₀ in untreated rats. Animals which survived poisoning with more than 800 mg/kg of Diazinon during the LD₅₀ study and which were treated with atropine and 2-PAMCl still showed marked signs of organophosphorus poisoning at 24 hours. However, almost all signs of poisoning had disappeared 48 hours after poisoning.

Table II. The Effects of Various Treatments on the LD₅₀ of Diazinon^a in Rats

| Treatment | | | | LD ₅₀ (95% Confidence Limits) mg/kg | LD ₅₀ Ratio Treated Untreated |
|-------------------------------|--------------------------------|------------------------|--------------|--|--|
| 1st (t = 10 min) ^b | 2nd (t = 4 hours) ^b | Atropine 16 mg/kg (im) | PAM 30 mg/kg | | |
| Atropine 16 mg/kg (im) | PAM 30 mg/kg | Atropine 16 mg/kg (im) | PAM 30 mg/kg | | |
| - | - | - | - | 293.8 (252.0-342.5) | 1.00 |
| + | - | - | - | 396.2 (301.8-520.5) | 1.35 |
| - | Oral | - | - | 513.4 (422.8-624.4) | 1.75 |
| - | iv | - | - | 355.0 (265.0-476.0) | 1.21 |
| + | iv | - | - | 498.8 (424.0-587.0) | 1.70 |
| + | Oral | - | - | 925.8 (716.1-1197.0) | 3.16 |
| + | Oral | + | - | 1082.0 (836.5-1398.0) | 3.69 |
| + | iv | + | - | 317.6 (241.0-419.5) | 1.08 |
| + | Oral | - | Oral | 832.0 (587.0-1170.0) | 2.84 |
| + | iv | - | Oral | 1000.0 (670.0-1489.0) | 3.41 |
| + | Oral | + | Oral | 794.1 (533.0-1180.0) | 2.71 |
| + | iv | + | Oral | 875.0 (718.0-1084.0) | 2.98 |
| + | iv | + | iv | 552.6 (315.5-612.0) | 1.88 |
| + | Oral | + | iv | 1000.0 (779.0-1278.0) | 3.41 |

^aDiazinon was given orally.

^bTime after poisoning with diazinon.

D. Reactivation of ChE by 2-PAMCl *In Vivo*.

Rats were poisoned with 0.8 LD₅₀ of Diazinon orally and treated with 2-PAMCl by the iv or oral route 24 hours later. One hour after this they were sacrificed and the ChE activity was measured. The results shown in table III indicate that treatment with 2-PAMCl reactivated the inhibited diaphragm ChE.

Table III. The Effect of 2-PAMCl^a on Diaphragm ChE Activity in Rats Poisoned With 0.8 LD₅₀ of Diazinon

| Treatment | Diaphragm ChE Activity % Normal ± S.D. |
|------------|---|
| None | 10.8 ± 3.0 |
| PAM (oral) | 35.3 ± 6.4 |
| PAM (iv) | 45.2 ± 7.5 |

^aPAM, 30 mg/kg, 24 hours after poisoning.

E. Blood ChE Activity and Survival in Rabbits Treated With Atropine and 2-PAMCl.

Poisoning of rabbits with 1600 mg/kg of Diazinon sc resulted in marked inhibition of whole blood ChE (figure 2). Administration of 2-PAMCl by the iv route alone (broken line) or with oral therapy (solid line) resulted in rapid reactivation of blood ChE. Within a short period muscle weakness reappeared in animals given 30 mg/kg of 2-PAMCl iv, and analysis of blood ChE showed renewed inhibition. Return of muscle weakness was delayed in those animals that received the oxime by both the oral and iv route. Blood ChE levels in the rabbits showing renewed signs of poisoning were again depressed. A second injection of 2-PAMCl again decreased muscle weakness and reactivated blood ChE. The first 2-PAMCl injection appeared to be more effective in reactivating inhibited blood ChE than subsequent injections (figure 2). All animals given only iv therapy were dead the next morning. Those that received 2-PAMCl by both the iv and oral routes were still alive at this time and were given 2-PAMCl iv. Reactivation of the blood ChE still occurred (figure 2, solid line).

IV. DISCUSSION.

The fraction of activity that can be restored to a phosphorylated enzyme decreases exponentially in the absence of an effective oxime.^{12,13,14} The rate of formation of the irreversibly inhibited ("aged") phosphorylated enzyme in brain increases in the order: diethyl phosphate < diisopropyl phosphate < dimethyl phosphate.^{15,16} Since animals poisoned with Diazinon would be expected to produce a diethyl phosphorylated enzyme, one would expect the rate of aging to be relatively slow. Our finding is in approximate agreement with that of Hobbiger¹⁵ who reported the half-time ($t_{1/2}$) for aging of the diethyl phosphoryl enzyme to be 36 hours. In our study the $t_{1/2}$ for aging of rat brain AChE from animals poisoned with Diazinon was 42 hours. This longer time interval may be due to the time required for metabolic activation of Diazinon to an active anticholinesterase.

Two factors are probably responsible for recovery of ChE activity in animals poisoned with organophosphates: (1) synthesis of new ChE and (2) spontaneous dephosphorylation of the inhibited ChE.¹⁷ A contribution from either source would not be measurable in cases of poisoning with indirect inhibitors like Diazinon until all of the insecticide had been converted to the active anticholinesterase. The persistence of the active inhibitor would be expected to be dose dependent; i.e., the higher the dose, the longer an "appreciable" concentration of active inhibitor would be present in the body. The comments apply directly to the findings in table I. Spontaneous reactivation of diaphragm ChE could not be detected during the first 24 hours, but both tissues underwent progressive recovery thereafter. Comparable spontaneous recovery of rat brain ChE has also been observed in animals poisoned with parathion and tetraethylpyrophosphate (TEPP).¹⁸ which, like Diazinon, form a diethyl phosphorylated enzyme.

Wills¹ evaluated some of the findings of Namba on the effectiveness of oximes in reactivating ChE and in reducing mortality in experimental animals after poisoning with alkylphosphates. 2-PAMCl in this instance was reported to have failed to reduce mortality caused by Diazinon poisoning. Wills pointed out that oximes were used alone in those experiments, and that if a mixture of atropine and oxime had been used for treatment of the poisoning by Diazinon, the results might have been different from those obtained. Our findings and those of others^{19,20} support this supposition. 2-PAMCl when administered orally in conjunction with atropine, im, increased the LD₅₀ to 3.16 times that in untreated rats (table II). When 2-PAMCl was administered iv adjunctively with atropine, the LD₅₀ was only increased 1.7 times. The difference between the protection offered by the administration of oral and iv 2-PAMCl in conjunction with atropine was probably caused by the greater duration of an effective level of oxime in the blood after oral 2-PAMCl treatment.⁷ The failure of 2-PAMCl when administered iv along with atropine to give protection significantly beyond atropine alone (table II) was probably because of its rapid loss from the blood plasma.⁸

Oral administration of 2-PAMCl shortly after Diazinon suggested the possibility of interaction between the two,^{21,22} which could possibly affect the protection ratio offered by oxime treatment. This possibility was studied by measuring the intraperitoneal (ip) LD₅₀'s of Diazinon alone and with atropine im and 2-PAMCl orally. They were found to be 399.0 (341.9 to 465.7) mg/kg and 1242.0 (928.0 to 1662.0) mg/kg, respectively, or 3.1 times the untreated LD₅₀.

The similarity in protection by oral 2-PAMCl against Diazinon administered orally or ip suggests that any direct interaction between orally administered oxime and Diazinon, if it occurs, makes little or no difference in the protection offered against this insecticide.

Failure to obtain antidotal action against some organophosphates which form an initially reactivatable phosphorylated ChE can arise from a difference in the times the active inhibitor and the oxime enter into the circulation. Evidence for this interpretation is shown in figure 2, which clearly indicates the necessity for maintaining an adequate level of oxime in the body for as long as the insecticide is being activated to a potent anticholinesterase. Therefore repeated doses of oxime are needed to maintain effective oxime levels in the body in cases of poisoning with Diazinon or other indirect inhibitors of ChE.^{23,24}

V. CONCLUSIONS.

Our findings suggest that effective treatment of Diazinon intoxication requires the maintenance of a level of oxime high enough to reactivate inhibited ChE during the time in which active inhibitor is formed. This may be more readily achieved when 2-PAMCl is given orally following an initial intravenous dose in conjunction with atropine given intramuscularly.

LITERATURE CITED

1. Wills, J. H. Recent Studies of Organic Phosphate Poisoning. *Federation Proceedings* 18, 1020-1025 (1959).
2. Sanderson, D. M., and Edson, E. F. Oxime Therapy in Poisoning by Six Organophosphorus Insecticides in the Rat. *J. Pharm. Pharmacol.* 11, 721-728 (1959).
3. Hobbiger, F. Effect of Nicotinhydroxamic Acid Methiodide on Human Plasma Cholinesterase Inhibited by Organophosphates Containing a Dialkylphosphoro Group. *Br. J. Pharmac. Chemother.* 10, 356-362 (1955).
4. Fallscheer, H. O., and Cook, J. W. Studies on the Conversion of Some Thionophosphates and a Dithiophosphate to *In Vitro* Cholinesterase Inhibitors. *J. Ass. Off. Agric. Chem. Wash.* 39, 691-697 (1956).
5. O'Brien, R. D. Activation of Thionophosphates by Liver Microsomes. *Nature* 183, 121-122 (1959).
6. Gage, J. C. A Cholinesterase Inhibitor Derived From *O,O*-Diethyl *O-p*-Nitrophenyl Thiophosphate *In Vivo*. *Biochem J.* 54, 426-430 (1953).
7. Zvirblis, P., and Kondritzer, A. Prophylaxis Against Sarin Poisoning in the Rat by Oral Administration of Pralidoxime Chloride. *J. Pharmacol. Exptl. Therap.* 157, 432-434 (1967).
8. Sundwall, A. Plasma Concentration Curves of *N*-Methylpyridinium-2-alkoxime Methane Sulphonate (P₂S) After Intravenous, Intramuscular and Oral Administration in Man. *Biochem. Pharmacol.* 5, 225-230 (1960).
9. Fleisher, J. H., Pope, E. J., and Spear, S. F. Determination of Red Blood Cell Cholinesterase Activity in Whole Blood. An Application of the Colorimetric Method to the Blood of the Rabbit, Rat, Pig, Dog, Goat, and Monkey. *A. M. A. Archs. Ind. Hlth.* 11, 332-337 (1955).
10. Thompson, W. R. Use of Moving Averages and Interpolation to Estimate Median-Effective Dose. *Bact. Rev.* 11, 115-145 (1947).
11. Weil, C. S. Tables for Convenient Calculation of Median-Effective Dose (LD₅₀ or ED₅₀) and Instructions in Their Use. *Biometrics* 8, 249-263 (1952).
12. Fleisher, J. H., and Harris, L. W. Dealkylation as a Mechanism for Aging of Cholinesterase After Poisoning with Pinacolyl Methylphosphonofluoridate. *Biochem. Pharmac.* 14, 641-650 (1965).
13. Harris, L. W., Fleisher, J. H., Clark, J., and Cliff, W. J. Dealkylation and Loss of Capacity for Reactivation of Cholinesterase Inhibited by Sarin. *Science* 154, 404-407 (1966).

14. Wilson, I. B., Ginsberg, S., and Meislich, E. K. The Reactivation of Acetylcholinesterase Inhibited by Tetraethyl Pyrophosphate and Diisopropyl Fluorophosphate. *J. Amer. Chem. Soc.* 77, 4286-4291 (1955).
15. Hobbiger, F. Protection Against the Lethal Effects of Organophosphates by Pyridine-2-aldoxime Methiodide. *Brit. J. Pharmacol.* 12, 438-446 (1957).
16. Witter, R. F., and Gaines, T. B. Rate of Formation *In Vivo* of the Unreactivable Form of Brain Cholinesterase in Chickens Given DDVP or Malathion. *Biochem. Pharmac.* 12, 1421-1427 (1963).
17. Davison, A. N. Return of Cholinesterase Activity in the Rat After Inhibition by Organophosphorus Compounds. *Biochem. J.* 60, 339-346 (1955).
18. DuBois, K. P., Doull, J., Salerno, P. R., and Coon, J. M. Studies on the Toxicity and Mechanism of Action of *p*-Nitrophenyl Diethyl Thionophosphate (Parathion). *J. Pharmacol. Exptl. Therap.* 95, 79-91 (1949).
19. Woodard, G. T. The Treatment of Organic Phosphate Insecticide Poisoning With Atropine Sulphate and 2-PAM (2-Pyridine Aldoxime Methiodide). *Vet. Med.* 52, 571-578 (1957).
20. Younger, E. L., and Radeleff, R. D. Use of Pyridine-2-Aldoxime Methochloride in the Treatment of Organic Phosphorus Compound Poisoning in Livestock. *Am. J. Vet. Res.* 25, 981-987 (1964).
21. Jandorf, B. J. Chemical Reactions of Nerve Gases in Neutral Solution. I. Reactions with Hydroxylamine. *J. Amer. Chem. Soc.* 78, 3686-3691 (1956).
22. Green, A. L., and Saville, B. The Reaction of Oximes With Isopropyl Methylphosphonofluoridate (Sarin). *J. Chem. Soc. PT4*, 3887-3892 (1956).
23. Namba, T., and Hiraki, K. PAM (Pyridine-2-Aldoxime Methiodide) Therapy for Alkylphosphate Poisoning. *J. A. M. A.* 166, 1834-1839 (1958).
24. Francis, J. I., and Barnes, J. M. Studies on the Mammalian Toxicity of Fenthion. *Bull. WHO.* 29, 205-212 (1963).

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| 13. ABSTRACT (U) This work was undertaken to determine the effectiveness of atropine-2-PAMCl treatment of animals poisoned with Diazinon, an organophosphate requiring metabolic activation prior to becoming an inhibitor of Cholinesterase (ChE). Inhibited diaphragm ChE in rats poisoned with this organophosphate and treated 24 hours later with 2-PAMCl showed significant reactivation regardless of whether the oxime was administered by the oral or intravenous route. However, 2-PAMCl given with intramuscular atropine to rats intoxicated with Diazinon was significantly more effective by the oral route than when injected intravenously. Our findings suggest that effective treatment of Diazinon intoxication requires the maintenance of a level of oxime high enough to reactivate inhibited ChE during the time in which active inhibitor is formed. This can be more readily achieved when 2-PAMCl is given orally, in conjunction with atropine given intramuscularly, following an initial intravenous dose. | | |
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